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Castration affects reproductive but not aggressive behavior in a cichlid fish

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ABSTRACT

Gonads are the main source of sex steroids, which have been implicated in the regulation of sexually differentiated behavior, such as reproductive and aggressive displays. In the Mozambique tilapia (*Oreochromis mossambicus*) territorial males have higher androgen levels than non-territorials, express reproductive behavior and use a urine-borne pheromone to signal their social status towards conspecifics. Here we investigated the effects of gonadectomy on the circulating levels of androgens and cortisol, and on the expression of aggressive and reproductive behavior (nest building, courtship behavior, and nuptial coloration). Males were either castrated, urine bladder damaged, or sham-operated and visually exposed to a group of females during 8 consecutive days and subsequently to a male on day 9. The urine bladder damaged treatment was included in the experimental design because a full castration procedure in this species causes quite often damage to the urine bladder. Gonadectomy lowers dramatically the circulating levels of androgens measured at 4 and 8 days post-castration and abolishes the expression of nest building, courtship behavior and nuptial coloration, but has no effect on the expression of aggressive behavior. These results confirm the gonads as the main source of androgens in this species and show that androgens are necessary for the expression of reproductive behaviors. However, the expression of aggressive behavior seems to be decoupled from gonadal steroids, namely androgens, suggesting the action of independent central mechanisms.

1. Introduction

The gonads are the main source of sex hormones, which have a pivotal role in sex differentiation, the development of secondary sex characters and the expression of sex specific behaviors (Nelson, 2005). Therefore, gonadal hormones have been viewed as playing an integrative role that assures the co-expression of sets of functional traits in the same phenotype, such that differentiation of the gonads into a given sex express implies the expression of the corresponding secondary sexual characters and behavior. In males, androgens play such a role, either directly or through aromatization into estrogens (Balthazart and Ball, 1998). Androgens have been implicated in the expression of multiple aspects of reproductive behavior including courtship displays and breeding-related aggression (e.g. defence of breeding territories or mates, Pfaff et al., 2008). However, the fact that androgens modulate the expression of sex-specific behavior does not demonstrate *per se* that they are necessary for its expression. In fact, a compilation of the effects of castration on reproductive and aggressive behavior across different fish species shows divergent results (see Oliveira and Gonçalves (2008), and Gonçalves and Oliveira (2010) for recent reviews). Castration reduced or abolished the expression of reproductive behaviors, such as nest building, nuptial coloration or courtship displays, in some species, whereas it had no effect in others (Oliveira and Gonçalves, 2008; Gonçalves and Oliveira, 2010). Similarly, the effects of castration on aggressive behavior ranged from negative, to neutral, and even to positive (Oliveira and Gonçalves, 2008; Gonçalves and Oliveira, 2010), and divergent results can be found within the same species (e.g. *Gasterosteus aculeatus*; Hoar, 1962; Baggerman, 1966; Wootton, 1970).

Cichlid fishes have been emerging as model organisms in behavioral neuroendocrinology, given the complexity of their social and breeding behavior (e.g. cooperative breeding in *Neolamprologus pulcher*, Desjardins et al., 2007; Fitzpatrick, 2005; Taves et al., 2009; Wong and Balshine, 2011) and the diversity of mating systems and parental care types present in closely related species

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that allows for comparative studies (e.g. phylogenetic test of the challenge hypothesis in African cichlids with divergent mating systems, Hirschenhauser et al., 2004). In our lab we have been using the Mozambique tilapia (*Oreochromis mossambicus*) as a model to study the neuroendocrinology of social behavior. In this species androgens respond to social interactions (Oliveira et al., 1996) and socially driven changes in androgens moderate the expression of both secondary sex characters (Oliveira and Almada, 1998) and male–male competitive behavior (i.e. winner effect, Oliveira et al., 2009). Therefore it became important to assess if androgens are necessary for the expression of reproductive and aggressive behaviors, which would suggest an activational role of androgens on the neural circuits underlying behavior, or if they act as moderators, which would be compatible with a neuromodulator role (e.g. facilitator) on a neural circuit that would be functional even in the absence of androgens.

Here the effects of castration on reproductive behavior (nuptial coloration, nest building and courtship displays) expressed in the presence of females, and on aggressive behavior displayed towards a neighboring male were tested in male *O. mossambicus*. Given that the complete excision of the gonads in this species involves damage to the urinary bladder (see details in the Methods Section 2.3), and the fact that male *O. mossambicus* use urine-born compounds to communicate social status (Almeida et al., 2005; Barata et al., 2007), the effects of urinary bladder damage were also investigated. In summary, the specific aims of this study were: (1) to characterize the effects of castration in circulating levels of androgens (testosterone, T; and 11-keto-testosterone, KT); (2) to investigate the effects of castration on reproductive and aggressive behaviors; and (3) to verify the effects of urinary bladder damage on circulating levels of androgens, and on aggressive and courtship behavior.

2. Materials and methods

2.1. Animals and housing

The tilapia males used in this study were part of a stock held at ISPA – Instituto Universitário (Lisboa, Portugal) that is maintained in glass tanks (120 × 40 × 50 cm, 240 L) with a fine gravel substrate, in stable social groups of 4 males and 5 females per tank. Tanks were supplied with a double filtering system (sand and external biofilter, Eheim) and constant aeration. Water quality was monitored on a weekly basis for nitrite (0.2–0.5 ppm), ammonia (<0.5 ppm) (Pallintest kit®) and pH (6.0–6.2). Fish were kept at a temperature of 26 ± 2 °C and a 12L:12D photoperiod, and fed with commercial cichlid floating and sinking sticks. The social status of the males in each tank, based on the body coloration and on the possession of a spawning pit on the substrate, was monitored on a daily basis.

2.2. Experimental protocol

Twenty-eight territorial males [territorial status was assessed from nuptial coloration and nest defence behavior; body mass (mean ± SD): castrated = 41.4 ± 7.0 g; sham = 41.7 ± 8.4 g; urinary bladder damaged = 33.3 ± 1.7 g] were removed from the stock tanks and introduced in experimental aquaria (47 cm × 24 cm × 30 cm, following stock conditions) where they were left for 2 days in order to familiarize with the new aquarium (days -2 and -1, see Fig. 1 for the time line of the general protocol). One to 3 h before males introduction in the experimental aquaria, 4 females [body mass (mean ± SD): 23.2 ± 5.2 g, $N = 30$)] were collected from stock tanks and introduced in an adjacent aquarium (70 cm × 37 cm × 30 cm) and acted as the visual stimulus to focal males. On day 0 males underwent one of three types of surgery: (1) sham operation; (2) castration; and (3) urinary bladder damage. The latter treatment was used as a complementary control treatment because the total removal of the testis implied damaging to the urinary bladder (Fig. 2). In order to prevent exposure of the experimental males to their own hormones, that might have been released into the water, the water of the experimental aquaria was renewed immediately after surgery and any nests built by the males on days -2, -1 and 0 (i.e. before surgery) were destroyed. Males were then returned to their experimental aquaria and kept in visual contact with females.

Blood was collected from the caudal vein (using 1 ml syringes with 25G/16 mm needles) under anaesthesia (MS-222, Pharmaq; 300–400 ppm) at days 0 (before surgery), 4 and 8. Blood sampling always took less than 4 min since the induction of anaesthesia, which prevents any possible effects of handling stress on cortisol levels (Foo and Lam, 1993). Blood was centrifuged and the plasma was stored at −20 °C until further processing.

At day 8 an unfamiliar male to the focal male was placed isolated in an adjacent aquarium (18 cm × 30 cm × 15 cm) at the opposite side of the females' aquarium (the side of the male and female aquaria was randomized across replicates). During day 8 visual access between the two males was prevented by placing a white opaque partition between the aquaria. During day 9 visual access to females was blocked by insertion of a white opaque partition and visual contact to the male in the adjacent aquarium was allowed for 15 min. The focal male behavior (bite, displays and courtship) towards the adjacent (putative intruder) male was

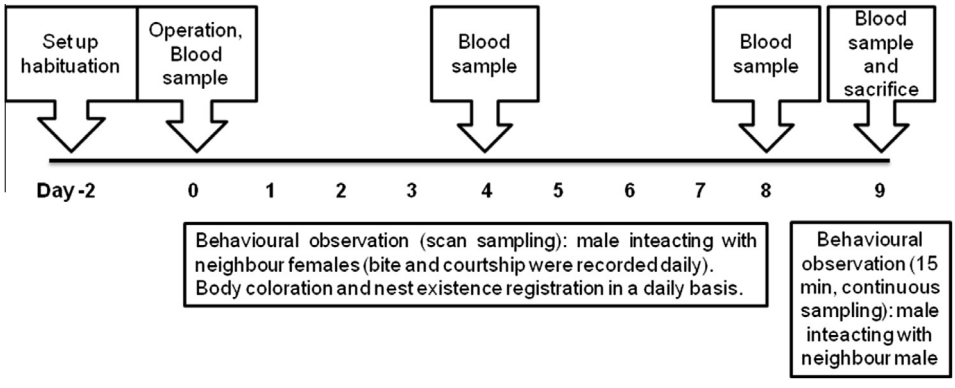

Fig. 1. Experimental design – focal males were introduced in the experimental set up 2 days before the operation for habituation. On day 0 males were operated creating sham, castrated or urine bladder damaged males. Male interaction with neighboring females was observed for 10 min each day. Nest presence and male nuptial coloration were registered daily. On the day 9, focal males were exposed to a territorial neighboring male and the behavior of the focal male was recorded for 15 min. Blood samples were collected on days 0 (before operation), 4 and day 8 – (after the interaction with the neighbor male).